Supplemental Figure 1. Effect of lapatinib on proliferation of human breast cells. Normal human mammary epithelial cells (HMECs) were obtained from Clonetics (San Diego, CA). MCF10A, BT474, MCF7, MDA-MB-468, and MDA-MB-231 were initially obtained from ATCC. HMECs were maintained in Mammary Epithelial Basal Medium (MEBM) supplemented with the Mammary Epithelial Growth Media (MEGM) kit (Cambrex Corporation, East Rutherford, NJ). BT474, MCF7, MCF10A, MDA-MB-468, and MDA-MB-231 cells were maintained in DMEM medium (Invitrogen) supplemented with 5% Fetal Bovine Serum. For growth assays, cells were seeded in 96-well plates at 1000 cells per well (MCF7, MCF10A, MDA-MB-468, MDA-MB-231) or 2000 cells per well (HMECs and T47D cells) followed by treatments with 0.1% of DMSO, or Lapatinib (GW572016) (1 pM–10 μM) for up to 10 days. Cell proliferation was measured using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Proliferation curves were plotted for: **A**) Normal mammary epithelial cells (HMECs) and immortal breast cells (MCF10A), **B**) Mammary tumors cells BT474 (overexpressing ErbB2), MCF7 (low epidermal growth factor receptor [EGFR] and ErbB2 expression), MDA-MB-468 (overexpressing EGFR), and MDA-MB-231 (low EGFR, ErbB2 expression). The number of viable cells was approximated by measuring the absorbance at 550 nm. Each point represents the mean of one experiment performed in eight replicate wells. *indicates statistically significantly different from vehicle (DMSO)-treated cells ($P < .05$, Student *t* test). Mean values are plotted; error bars correspond to 95% confidence intervals.

Supplemental Figure 2. Comparison of the histological features and the expression of the erbB2 transgene in normal and malignant mammary tissues from vehicle- and lapatinib-treated mice. MMTV-erbB2 mice were randomly assigned to receive treatment with vehicle or lapatinib (75 mg/kg body weight) (n = 20 mice per group) for 6 days each

week for 5 months starting at 3 months of age. All mice were sacrificed by asphyxiation in a carbon dioxide chamber after 5 months of treatment. Both tumor and normal mammary tissues were immediately resected. Tissue samples were fixed in 4% phosphate-buffered formaldehyde overnight and then embedded in paraffin for histology examination and biomarker measurement. Mammary tissue sections were stained with hematoxylin–eosin (H&E) or with a rabbit polyclonal antibody specific for ErbB2 (c-ErbB2 Ab-1, Neomarkers; 1:50) using immunohistochemical staining (the primary antibody was detected using a secondary antibody (a biotinylated goat anti-rabbit IgG) and developed with the DAB chromagen using the Vector ABC kit (from Vector Laboratories, Burlingame, CA), (shown as **brown staining**). H & E and ErbB2 staining was done in 20 vehicle- and 20 lapatinib-treated mice. Representative fields of normal mammary gland and tumor tissue from one vehicle-treated mouse and from one lapatinib-treated mouse are shown **[AU: edit OK?]**.

Supplemental Figure 3. Transcription of cyclin D1, epiregulin, and p27 in vehicle (DMSO) and lapatinib-treated normal human mammary epithelial cells (HMECs). Total RNA was extracted from HMECs treated with lapatinib as described in Supplementary Figure 1 at 6, 12, and 24 hours after epidermal growth factor (EGF) stimulation and used as template in quantitative real-time reverse transcription-polymerase chain reaction assays specific for human cyclin D1, epiregulin, and p27 (41–43). The values plotted are mean relative mRNA expression level (normalized using cyclophilin mRNA levels for each sample) of vehicle-treated samples versus lapatinib-treated samples from three independent experiments, each with samples in triplicate. Error bars correspond to 95% confidence intervals. *P* values (two-sided) are from two-way ANOVA.